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September 9, 2005

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Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Art Unit 1644

Re: U.S. Utility Patent Application
Application No. 08/474,388; Filed: June 7, 1995
For: **ICAM-1 Preparation**
Inventor: SPRINGER *et al.*
Our Ref: 1011.004000D/RCM/GLL

Sir:

In response to the Notification of Non-Compliant Appeal Brief (37 C.F.R. § 41.37) mailed August 10, 2005, Applicants submit the following documents for appropriate action by the U.S. Patent and Trademark Office:

1. Communication with attachment;
2. Copy of Office Action mailed December 15, 2000;
3. Copy of date stamped postcard filed on October 24, 2000;
4. Copy of Supplemental Amendment Under 37 C.F.R. § 1.111 filed on October 24, 2000; and
5. One return postcard.

It is respectfully requested that the attached postcard be stamped with the date of filing of these documents, and that it be returned to our courier. In the event that extensions of time are necessary to prevent abandonment of this patent application, then such extensions of time are hereby petitioned.

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Gaby L. Longsworth

Gaby L. Longsworth
Attorney for Applicants
Registration No. 47,756

RCM/GLL/eaf
Enclosures

440898v1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
SPRINGER *et al.*
Appl. No.: 08/474,388
Filed: June 7, 1995
For: **ICAM-1 Preparation**

Confirmation No.: 2682
Art Unit: 1644
Examiner: P. Gambel
Atty. Docket: 1011.004000D/RCM/GLL

Communication

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

Applicants received a Notice of Non-Compliant Appeal Brief Under 37 C.F.R. § 41.37 mailed August 10, 2005. According to the Examiner, the Appeal Brief filed on October 21, 2002, is defective for failure to comply with one or more provisions of 37 CFR § 41.37. Apparently, a copy of a Supplemental Amendment filed October 24, 2000, is missing from the USPTO's records, even though a transmittal sheet indicating the submission of the Supplemental Amendment is noted. The Examiner requested that Applicants provide a copy of the Supplemental Amendment filed October 24, 2000, including the stamped postcard receipt, as well as a copy of the Office Action dated December 5, 2000, which is also missing from the USPTO's records.

In response to the Examiner's request, Applicants provide herewith a copy of the Supplemental Amendment filed October 24, 2000, including a copy of the stamped postcard receipt. Applicants did not locate an Office Action mailed December 5, 2000; however, a copy of the Office Action dated December 15, 2000 (Paper No. 28) is provided.

In response to the Examiner's comments regarding pending claims 71, 72, 77 and 78, as well as claims 73 and 76, Applicants provide herewith, as a courtesy to the Examiner, a chart detailing the various amendments to the claims and the date the amendments were filed. Applicants note that there is a discrepancy in claim 73. In the amendment filed on October 15, 1997, claim 73 was amended as:

73. The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 can specifically bind LFA-1.

However, claim 73 should have been properly amended as follows:

73. The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 can specifically bind ~~lymphocytes~~ LFA-1.

Applicants respectfully request that, if necessary, the Examiner make the appropriate correction to claim 73 pursuant to 37 C.F.R. § 1.121(g).

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Gaby L. Longsworth

Gaby L. Longsworth
Attorney for Applicants
Registration No. 47,756

Date: September 9, 2005

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440901v1

Claim	As filed	Once amended	Twice amended	Thrice amended	As pending
71[1]	A purified or isolated ICAM-1 preparation substantially free of natural contaminants, wherein said purified or isolated ICAM-1 exhibits at least one biological activity of native ICAM-1. (Filed Jan. 7, 1997)	A purified or isolated ICAM-1 preparation substantially free of natural contaminants, wherein said purified or isolated ICAM-1 [exhibits at least one biological activity of native ICAM-1] is capable of binding to LFA-1, Mac-1, or p150,95. (Amendment filed July 6, 1998)	A purified or isolated ICAM-1 preparation substantially free of natural contaminants, wherein said purified or isolated ICAM-1 is derived from human cells or tissues and is capable of binding to LFA-1, Mac-1, or p150,95. (Amendment filed May 24, 1999)	A purified or isolated ICAM-1 preparation substantially free of natural contaminants, wherein said purified or isolated ICAM-1 is derived from human cells or tissues and is capable of binding to LFA-1, Mac-1, or p150,95; and wherein said purified or isolated ICAM-1 binds with specificity to the ICAM-1 monoclonal antibody RR1/1. (Amendment filed Oct. 24, 2000)	A purified or isolated ICAM-1 preparation substantially free of natural contaminants, wherein said purified or isolated ICAM-1 is derived from human cells or tissues and is capable of binding to LFA-1, Mac-1, or p150,95; and wherein said purified or isolated ICAM-1 binds with specificity to the ICAM-1 monoclonal antibody RR1/1.
72	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 can bind LFA-1. (Filed Jan. 7, 1997)				The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 can bind LFA-1.
73	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 can bind lymphocytes. (Filed Jan. 7, 1997)	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 can specifically bind LFA-1. (Amendment filed Oct. 15, 1997)			The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 can specifically bind LFA-1.

[1] Claims 1-15, 24-26, and 45-68 were cancelled on June 7, 1995. Claims 16-23, 27-44 and 69-70 were cancelled on January 7, 1997.

Claim	As filed	Once amended	Twice amended	Thrice amended	As pending
74	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 can bind human rhinovirus. (Filed Jan. 7, 1997)	cancelled (Amendment filed July 6, 1998)	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 can bind [human rhinovirus] p150,95. (Amendment erroneously filed May 24, 1999)		
75	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is human spleen ICAM-1 having a molecular weight from about 72 kDa to about 91 kDa. (Filed Jan. 7, 1997)	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is human spleen ICAM-1 having a molecular weight [from about] of 72 [kDa] to [about] 91 kDa as determined by SDS polyacrylamide gel electrophoresis. (Amendment filed July 6, 1998)			The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is human spleen ICAM-1 having a molecular weight of 72 to 91 kDa as determined by SDS polyacrylamide gel electrophoresis.

Claim	As filed	Once amended	Twice amended	Thrice amended	As pending
76	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is ICAM-1 of JY cells having a molecular weight from about 76.5 kDa to about 97 kDa. (Filed Jan. 7, 1997)	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is ICAM-1 of JY cells having a molecular weight [from about] of 76.5 [kDa] to [about] 97 kDa as determined by SDS polyacrylamide gel electrophoresis. (Amendment filed July 6, 1998)	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is ICAM-1 of JY B-lymphoblastoid cells having a molecular weight of 76.5 to 97 kDa as determined by SDS polyacrylamide gel electrophoresis. (Amendment filed Oct. 24, 2000)		The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is ICAM-1 of JY B-lymphoblastoid cells having a molecular weight of 76.5 to 97 kDa as determined by SDS polyacrylamide gel electrophoresis.

Claim	As filed	Once amended	Twice amended	Thrice amended	As pending
77	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is ICAM-1 of myelomonocytic cell line having a molecular weight of about 114 kDa. (Filed Jan. 7, 1997)	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is ICAM-1 of myelomonocytic cell line having a molecular weight of [about] 114 kDa as determined by SDS polyacrylamide gel electrophoresis. . (Amendment filed July 6, 1998)			The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is ICAM-1 of myelomonocytic cell line having a molecular weight of 114 kDa as determined by SDS polyacrylamide gel electrophoresis.
78[2]	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is fibroblast ICAM-1 having a molecular weight of about 97 kDa. (Filed Jan. 7, 1997)	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is fibroblast ICAM-1 having a molecular weight of [about] 97 kDa as determined by SDS polyacrylamide gel electrophoresis. (Amendment filed July 6, 1998)			The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is fibroblast ICAM-1 having a molecular weight of 97 kDa as determined by SDS polyacrylamide gel electrophoresis.

[2] Claim 79 was filed on January 7, 1997, and cancelled on May 24, 1999.

Claim	As filed	Once amended	Twice amended	Thrice amended	As pending
80	A lipid membrane comprising isolated or purified ICAM-1 substantially free of natural protein contaminants, wherein said isolated or purified ICAM-1 is in a biologically active form. (Filed Jan. 7, 1997)	A lipid membrane comprising isolated or purified ICAM-1 substantially free of natural protein contaminants, wherein said isolated or purified ICAM-1 is [in a biologically active form] capable of binding to LFA-1, Mac-1, or p150,95. (Amendment filed July 6, 1998)	[A] An artificial lipid membrane comprising [isolated or] purified or isolated ICAM-1 [substantially free of natural protein contaminants], wherein said [isolated or] purified or isolated ICAM-1 is derived from human cells or tissues, is substantially free of natural protein contaminants in said artificial lipid membrane, and is capable of binding to LFA-1, Mac-1, or p150,95. (Amendment filed May 24, 1999)	An artificial lipid membrane comprising purified or isolated ICAM-1, wherein said purified or isolated ICAM-1 is derived from human cells or tissues, is substantially free of natural protein contaminants in said artificial lipid membrane, and is capable of binding to LFA-1, Mac-1, or p150,95; and wherein said purified or isolated ICAM-1 binds with specificity to the ICAM-1 monoclonal antibody RR1/1. (Amendment filed June 15, 2001)	An artificial lipid membrane comprising purified or isolated ICAM-1, wherein said purified or isolated ICAM-1 is derived from human cells or tissues, is substantially free of natural protein contaminants in said artificial lipid membrane, and is capable of binding to LFA-1, Mac-1, or p150,95; and wherein said purified or isolated ICAM-1 binds with specificity to the ICAM-1 monoclonal antibody RR1/1.

Claim	As filed	Once amended	Twice amended	Thrice amended	As pending
81	The lipid membrane as claimed in claim 80, wherein said ICAM-1 exhibits at least one biological activity selected from the group consisting of: LFA-1 binding, lymphocyte binding, and human rhinovirus binding. (Filed Jan. 7, 1997)	The lipid membrane as claimed in claim 80, wherein said ICAM-1 [exhibits] specifically binds to at least one [biological activity] ligand selected from the group consisting of: LFA-1 [binding], a lymphocyte [binding], and human rhinovirus [binding]. (Amendment filed Oct. 15, 1997)	The lipid membrane as claimed in claim 80, wherein said ICAM-1 specifically binds to [at least one ligand selected from the group consisting of:] LFA-1. (Amendment filed July 6, 1998)	[A] An artificial lipid membrane as claimed in claim 80, wherein said purified or isolated ICAM-1[specifically] binds to LFA-1. (Amendment filed May 24, 1999)	An artificial lipid membrane as claimed in claim 80, wherein said purified or isolated ICAM-1 binds to LFA-1.
82[3]	The lipid membrane as claimed in claim 80, wherein said lipid membrane is an artificial planar membrane. (Filed Jan. 7, 1997)				The lipid membrane as claimed in claim 80, wherein said lipid membrane is an artificial planar membrane.

[3] Claim 83 was filed on January 7, 1997, and cancelled on May 24, 1999. Claims 84-86 were filed on January 7, 1997, and cancelled on October 15, 1997. Claims 87-98 were filed on October 15, 1997, and cancelled on May 24, 1999.

Claim	As filed	Once amended	Twice amended	Thrice amended	As pending
99	The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 can bind p150,95. (Amendment filed Feb. 4, 2000. The claim was erroneously numbered as claim 83 but was corrected by the Examiner in an Advisory Action mailed February 18, 2000.)				The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 can bind p150,95.

Applicants: SPRINGER *et al.*

Art Unit: 1644

Examiner: Gambel, P.

Application No.: 08/474,388

Docket: 1011.004000D

Filed: June 7, 1995

Atty: SLF/RCM

For: **ICAM-1 Preparations**

When receipt stamp is placed hereon, the USPTO acknowledges receipt of the following documents:

1. PTO Transmittal Letter (*in duplicate*);
2. Supplemental Amendment Under 37 C.F.R. § 1.111;
3. An executed Declaration of Robert Rothlein Under 37 C.F.R. § 1.132 (with attached Exhibits A-H); and
4. One return postcard.

Please Date Stamp And Return To Our Courier

Hand Carry to Examiner P. Gambel
Art Unit: 1644

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

SPRINGER *et al.*

Appl. No. 08/474,388

Filed: June 7, 1995

For: **ICAM-1 Preparations**

Art Unit: 1644

Examiner: Gambel, P.

Atty. Docket: 1011.004000D/SLF/RCM

Supplemental Amendment Under 37 C.F.R. § 1.111

Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Examiner interview on October 3, 2000 (Paper No. 24), Applicants submit the following Amendments and Remarks.

Amendments

Please rewrite the following claims:

71. (Thrice Amended) A purified or isolated ICAM-1 preparation substantially free of natural contaminants, wherein said purified or isolated ICAM-1 is derived from human cells or tissues and is capable of binding to LFA-1, Mac-1, or p150,95; and

wherein said purified or isolated ICAM-1 binds with specificity to the ICAM-1 monoclonal antibody RR1/1.

76. (Twice Amended) The purified or isolated ICAM-1 preparation as claimed in claim 71, wherein said purified or isolated ICAM-1 is ICAM-1 of JY B-lymphoblastoid cells having a molecular weight of 76.5 to 97 kDa as determined by SDS polyacrylamide gel electrophoresis.

Remarks

Claims 71-73, 75-78, 80-82 and 99 are pending in the application, with claims 71 and 80 being the independent claims.

Applicants thank the Examiner for the courtesy extended to Applicants' representatives during the Examiner interview on October 3, 2000, in which the Examiner suggested certain changes to the language of claims 71 and 76. In particular, the Examiner suggested amending claim 71 to indicate that the purified or isolated ICAM-1 binds with specificity to the ICAM-1 monoclonal antibody RR1/1, to overcome the rejection under 35 U.S.C. § 112, first paragraph. Support for this amendment can be found in priority Appl. No. 07/045,963, *inter alia*, at pages 38-43 (Examples 8 and 9).

In addition, as the Examiner suggested, Applicants have amended claim 76 to indicate that JY cells are B-lymphoblastoid cells. Support for this amendment can be found in priority Appl. No. 07/045,963, *inter alia*, at page 15, lines 5-18; at page 19, lines 29-32; and at page 55, lines 17-21.

Applicants further provide the following remarks regarding the 35 U.S.C. §§ 102 and 103 rejections over Tomassini, thesis 8624033 (1986) [hereinafter "the Tomassini thesis"], and Tomassini *et al.*, *J. Virol. 58*:290-295 (1986) [hereinafter "the Tomassini article"]. The authors

of the Tomassini thesis and article indicated that Tomassini's purified HRRP (ICAM-1) receptor preparation is not able to bind HRV. (Tomassini thesis at 116, line 22, to 117, line 1; and Tomassini article at 295, col. 1, lines 20-25.) Since the binding sites for HRV and LFA-1 overlap, one of ordinary skill in the art would expect that any disruption in structure from the purification procedure leading to the elimination of HRV binding, would also reduce or eliminate LFA-1 binding.

Furthermore, this inability of Tomassini's HRRP preparation to bind HRV is not due to chemical denaturation and subsequent disruption of the binding sites on the virus by radio labeling protocols. The authors of the Tomassini thesis and article show that radio labeled HRV is capable of binding to membranes. These results indicate that the binding sites on radio labeled HRV are functional. Since radio labeled HRV binds membranes but does not bind to Tomassini's HRRP preparation, it is more likely than not that the binding sites on Tomassini's HRRP preparation are disrupted and not functional. As discussed with the Examiner during the interview, Applicants submit herewith a Declaration of Robert Rothlein Under 37 C.F.R. §1.132 in support of these conclusions. The Examiner indicated during the interview that in light of Tomassini's results and the Declaration of Robert Rothlein, the rejections under 35 U.S.C. §§ 102 and 103 are likely to be withdrawn.

Applicants submit that the amendments presented herein comply with the Examiner's suggestions. Applicants note that the Examiner's suggestions were not made in view of any newly discovered references. In view of the above amendments and remarks, and the Declaration of Robert Rothlein Under 37 C.F.R. §1.132, reconsideration of this Application is respectfully requested.

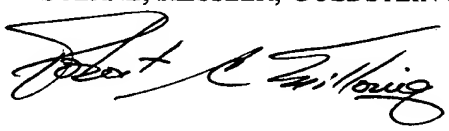
Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. It is believed that the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Supplemental Amendment is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



Robert C. Millonig
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Registration No. 34,395

Date: October 24, 2000

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08/474388

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
08/474,388	06/07/95	SPRINGER	T 1011.0040000

EXAMINER

HM22/1215

SAMUEL L FOX
STERNE KESSLER GOLDSTEIN & FOX
1100 NEW YORK AVENUE NW STE 600
WASHINGTON DC 20005-3934

ART UNIT	PAPER NUMBER
1644	28

DATE MAILED: 12/15/00

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

DEC 18 2000

☑ Responsive to communication(s) filed on 10/11/00

☐ This action is **FINAL**.

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☑ Claim(s) 71-73, 75-78, 80-82, 99 is/are pending in the application.
Of the above, claim(s) _____ is/are withdrawn from consideration.
☐ Claim(s) _____ is/are allowed.
☑ Claim(s) 71-73, 75-78, 80-82, 99 is/are rejected.
☐ Claim(s) _____ is/are objected to.
☐ Claim(s) _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.
☐ The specification is objected to by the Examiner.
☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.
☐ received in Application No. (Series Code/Serial Number) _____.
☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☑ Notice of Reference Cited, PTO-892
☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____
☐ Interview Summary, PTO-413
☐ Notice of Draftperson's Patent Drawing Review, PTO-948
☐ Notice of Informal Patent Application, PTO-152

DOCKETED Response due March 15, 2001
Start Day June 15, 2001

--SEE OFFICE ACTION ON THE FOLLOWING PAGES--

★ U.S. GPO: 1998-421-632/40206

DETAILED ACTION

1. Since this application is eligible for the transitional procedure of 37 CFR 1.129(a), and the fee set forth in 37 CFR 1.17(r) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.129(a).

Applicant's second submission after final filed on 9/5/00 (Paper No. 23) has been entered.

Applicant's amendment, 10/12/00 (Paper No. 26), is acknowledged.
Claims 71 and 76 have been amended.

Claims 71-73, 75-78, 80-82 and 99 are pending and being acted upon presently

Claims 1-70, 74, 79, 83-98 have been canceled previously.

2. Formal drawings and photographs have been submitted which fail to comply with 37 CFR 1.84. Please see the form PTO-948 previously sent in Paper No. 6.

3. The application is required to be reviewed and all spelling, TRADEMARKS, and like errors corrected.

Trademarks should be capitalized or accompanied by the ™ or ® symbol wherever they appear and be accompanied by the generic terminology. Although the use of trademarks is permissible in patent applications, the proprietary nature of the trademarks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks.

Appropriate corrections are required

4. The following is a quotation of the first paragraph of 35 U.S.C. § 112:
The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 80-82 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for "purified or isolated ICAM-1" which is bound by the RR-1 (see deposit requirement under 35 USC 112, first paragraph, herein) or defined by the amino acids of Figure 8; does not reasonably provide enablement for any "purified or isolated ICAM". The specification does not enable any person skilled in the art to which it pertains, or with which it is most clearly connected, to make and use the invention commensurate in scope with these claims.

Applicant has not provided sufficient biochemical information (e.g. molecular weight, amino acid composition, N-terminal sequence, etc.) that distinctly identifies ICAM other than ICAM bound by the RR-1 (see deposit requirement under 35 USC 112, first paragraph, herein) or defined by the amino acids of Figure 8.

"It is not sufficient to define the recombinant molecule by its principal biological activity, e.g. having protein A activity, because an alleged conception having no more specificity than that is simply a wish to know the identity of any material with that biological property." Colbert v. Lofdahl, 21 USPQ2d, 1068, 1071 (BPAI 1992).

While ICAM is an adhesion molecule which is capable of binding to LFA-1, MAC-1 or p150,95; there are a number of distinct adhesion molecules as well as distinct ICAM molecules, which encompass overlapping structural and functional attributes.

Reasonable correlation must exist between the scope of the claims and scope of enablement set forth. Without sufficient guidance, making and using ICAM-1 with the functional adhesion molecule properties at the time the invention was made, while providing or maintaining the claimed activity would be unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue in the absence of defining biochemical or enabling information.

Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. In re Van Geuns, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

6. Claims 71-73, 75-78, 80-82 and 99:

It is apparent that the RR1/1 antibody is required to practice the claimed invention. As a required element, it must be known and readily available to the public or obtainable by a repeatable method set forth in the specification. If it is not so obtainable or available, the enablement requirements of 35 USC 112, first paragraph, may be satisfied by a deposit of the cell line / hybridoma which produces this antibody. See 37 CFR 1.801-1.809.

In addition to the conditions under the Budapest Treaty, applicant is required to satisfy that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent in U.S. patent applications.

Amendment of the specification to recite the date of deposit and the complete name and address of the depository is required. As an additional means for completing the record, applicant may submit a copy of the contract with the depository for deposit and maintenance of each deposit.

If the original deposit is made after the effective filing date of an application for patent, the applicant should promptly submit a verified statement from a person in a position to corroborate the fact, and should state, that the biological material which is deposited is a biological material specifically identified in the application as filed, except if the person is an attorney or agent registered to practice before the Office, in which the case the statement need not be verified. See MPEP 1.804(b).

Applicant should note that the limitation of the RR/1 antibody is set forth in the scope rejection under 35 USC 112, first paragraph above.

If applicant amends the claims to rely upon the sequence in Figure 8; then the deposit requirement for the RR/1 antibody would be obviated.

7. Claim 75-78 and 80-82 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A) At the time the invention was made or priority relied upon (e.g. USSN 07/405,963, filed 5/4/87); claims 80-82 are indefinite in that they only describe the compositions of interest by an arbitrary protein name. While the name itself may have some notion of the activity of the protein, there is nothing in the claims which distinctly claims the protein and variants thereof. For example, others in the field may isolate the same protein and give such an entirely different name. Applicant should particularly point out and distinctly claim "ICAM-1" by claiming characteristics associated with the protein (e.g. amino acid composition, specifically bound by a deposited antibody,; e.g. RR/1). Claiming biochemical molecules by a particular name given to the protein by various workers in the field fails to distinctly claim what that protein is and what the compositions are made up of.

B) With respect to claims 75-78 and in consideration of the discrepancies often encountered in the art between protein molecular weight when determined by different methods, when a molecular weight is recited to characterize a protein the claims should include not only the method by which it was determined, e.g. whether by sodium dodecyl sulphate polyacrylamide gel electrophoresis, gel filtration or some other method, but also whether the determination was made under denaturing or non-denaturing conditions and whether reducing or non-reducing conditions were are used.

C) Applicant should specifically point out the support for any amendments made to the disclosure. See MPEP 714.02 and 2163.06

8. The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CAR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103© and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

10. Claim 71-73, 75-78, 80-82 and 99 are rejected under 35 U.S.C. § 102(b) as being anticipated by Tomassini (PhD Dissertation, 1986; of record) for the reasons of record.

Tomassini teaches the isolation and characterization of the human rhinovirus receptor, including various cell and membrane preparations (see entire document).

11. Claim 71-73, 75-78, 80-81 and 99 are rejected under 35 U.S.C. § 102(b) as being anticipated by Tomassini et al. (J. Virol. 58: 290-295, 1986; of record)for the reasons of record.

Tomassini teaches the isolation characterization of the human rhinovirus receptor, including cellular and membrane preparations (see entire document).

12. Claim 71-73, 75-78, 80-81 and 99 are rejected under 35 U.S.C. § 102(b) as being anticipated by Colonno et al. (Virus Attachment and Entry into Cells, Proceedings of an ASM Conference held in Philadelphia, PA, April 10-13, 1985).

Colonno et al. Teach the characterization of the cellular receptor specific for attachment of most human rhinovirus serotypes, including cellular and membrane preparations (see entire document, including pages 112-115).

Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced rhinovirus receptor.

The products of the instant claims and the prior art are defined in terms of physical characteristics. Comparison of the instant products with prior art is difficult since the Office is not equipped to manufacture the claimed product and/or prior art products that appear to be related and conduct comparisons. Also, it is noted that differences or variations were known in the art at the time the invention was made when protein molecular weight was determined by different methods and conditions.

The burden is on the applicant to establish a patentable distinction between the claimed and referenced products. See In re Best, 195 USPQ 430, 433 (CCPA 1977); In re Marosi, 218 USPQ 289, 292-293 (Fed. Cir. 1983); In re Fitzgerald et al., 205 USPQ 594 (CCPA 1980).

13. Claims 71-73, 75-78, 80-81 and 99 80-82 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Tomassini (PhD Dissertation, 1986; of record) AND/OR Tomassini et al. (J. Virol. 58: 290-295, 1986; of record) AND/OR Colonno et al. (Virus Attachment and Entry into Cells, Proceedings of an ASM Conference held in Philadelphia, PA, April 10-13, 1985) OR Tomassini (PhD Dissertation, 1986; of record) in view of the art known use of artificial membranes for a variety of uses in protein chemistry at the time the invention was made and to isolate and produce functional active proteins.

Tomassini (PhD Dissertation, 1986), Tomassini et al. (J. Virol., 1986) and Colonno et al. (Virus Attachment and Entry into Cells) are all taught above.

These references do not teach the use of artificial lipid membranes per se.

However, providing proteins of interest in artificial lipid membranes in a variety of means for a variety of purposes for the characterization and determination of the structure-function of a protein of interest was well known and practiced at the time the invention was made.

Also, it is noted that "artificial lipid membranes" has broad meaning; given the prosecution of the instant application and applicant's assertion that is irrelevant whether HRRP or ICAM-1 when associated with detergents meets the claimed limitation of artificial lipid membranes (see applicant's amendment filed 2/4/00; Paper No. 20; page 6).

It is noted the prior art teaches the isolation and characterization of the rhinovirus receptor which reads on the claimed ICAM-1 preparations.

Given applicant's arguments that the prior art isolated prior art rhinovirus receptor may not have the properties of binding LFA-1/Mac-1/p150,95; it is noted that prior art rhinovirus receptor is clearly identified as being the receptor for rhinovirus receptor.

Given this clear teaching and the clear motivation of the ordinary artisan to characterize this protein further, as taught by the each reference; the ordinary artisan would have been able to isolate and characterize the HRV receptor with the known and desired functional properties, such as HRV binding.

The reason or motivation to modify the reference may often suggest what the inventor has done, but for a different purpose or to solve a different problem. It is not necessary that the prior art suggest the combination to achieve the same advantage or result discovered by applicant. See MPEP 2144.

Although the prior art may not known that the HRV receptor also had the ability to bind to LFA-1/Mac-1/p150,95; these adhesion molecule properties would have been expected properties given the isolation of a functional HRV receptor with ability to bind HRV.

One of ordinary skill in the art at the time the invention was made would have been motivated to isolate and characterize the structure-function nature of the HRV receptor, including the art known use of artificial lipid membranes; given its clear importance in rhinovirus attachment and infection. From the teachings of the references, it was apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

14. Applicant's arguments in conjunction with the Rothlein Declaration under 37 CFR 1.132, 10/12/00 (Paper Nos. 26/27), have been fully considered but not found convincing.

Applicant argues in conjunction with the Rothlein Declaration that the Tomassini purified HRRP is not able to bind HRV (e.g. see page 46 of the Tomassini PhD Thesis).

It is noted that any disruption in structure from the purification procedure leading to the elimination of HRV binding would also reduce or eliminate LFA-1 binding.

However, it is noted that in characterizing the HRV receptor, Tomassini et al. teach the isolation of the cellular receptors can be achieved by several methods, including but not limited to detergent treatment (page 113). Tomassini et al. clearly teach that the vast number of HRV serotypes use this HRV receptor for attachment, as determined by competition and functional assays (page 113).

While the thesis indicates that repeated attempts to use radiolabeled HRV in place of receptor antibody in the RIA gave inconclusive results owing to poor virus binding; it is not clear the conditions of the assay or the functional attributes of the radiolabeled receptor, since the data or details are not provided (page 44, paragraph 1).

However, immunoaffinity purification did further purify the HRV receptor, wherein said HRV receptor was bound by specific antibody, wherein said anti-HRV antibody could block HRV attachment and that the HRV receptor could be used as an immunogen to generate antisera which selectively inhibit HRV attachment to susceptible cells tested by both membrane binding and cell protection assays (pages 50-69).

Therefore, the thesis clearly states that the HRV receptor is utilized by the major groups of HRVs during attachment to cells (page 65, and Discussion on pages 107-118). Additional biochemical studies (pages 69-83) as well as initial cloning of the HRV receptor (pages 83-105) are also disclosed.

Further, applicant has failed to rebut prima facie showing of inherency or obviousness absent objective evidence such as side-by-side testing that would address the ability of the prior art HRV receptors ability to bind LFA-1/Mac-1/p150,95. See Ex parte Raske, 28 USPQ2d 1304 (BPAI 1993).

Even if there is an indication that there may be reduced binding of a particular radiolabeled HRV receptor preparation reduced binding to HRV; it maintained the ability to bind.

Further, this does not address the ability of the prior HRV receptor ability to bind LFA-1/Mac-1/p150,95

Although applicant in conjunction with the Rothlein declaration distinguish the cloning disclosed in the Tomassini thesis from that relied upon Tomassini et al. (PNAS 86: 4907-4911, 1989); it is clear that the Tomassini thesis as well as the other references clearly teach that the HRV receptor is indeed the receptor for rhinovirus, that the HRV receptor is bound by antibodies that block HRV attachment or binding, and that the HRV receptor can be used as an immunogen to produce an antibody that blocks HRV attachment and binding.

Either it was inherent or expected at the time the invention was made that the HRV receptor identified and characterized by the references had the ability to bind virus and, in turn, would have either the inherent or expected properties of binding LFA-1/Mac-1/ p150,95.

Products of identical chemical composition can not have mutually exclusive properties. A chemical composition and its properties are inseparable. Therefore, if the prior art teaches the identical chemical structure, the properties applicant discloses and/or claims are necessarily present. In re Spada 15 USPQ2d 1655, 1658 (Fed. Cir. 1990). See MPEP 2112-2113, including 2112.01.

Also, see Mehl/Biophile International Corp. V. Milgraum, 52 USPQ2d 1303 (Fed. Cir. 1999); Atlas Powder Co. V. IRECO, 51 USPQ2d 1943 (Fed. Cir. 1999).

For example, Atlas Powder Co. V. IRECO, 51 USPQ2d 1943 (Fed. Cir. 1999) states:
"Artisans of ordinary skill may not recognize the inherent characteristics or functioning of the prior art... However, the discovery of a previously unappreciated property of a prior art composition, or of a scientific explanation for the prior art's functioning, does not render the old composition patentably new to the discoverer. " The Court further held that "this same reasoning holds true when it is not a property but an ingredient which is inherently contained in the prior art".

The reason or motivation to modify the reference may often suggest what the inventor has done, but for a different purpose or to solve a different problem. It is not necessary that the prior art suggest the combination to achieve the same advantage or result discovered by applicant. See MPEP 2144.

While the LFA-1/Mac-1/p150,95 binding of the HRV receptor was not disclosed; the prior art need not disclose a newly discovered property in order for a prima facie case of obviousness. If the claimed and the structurally similar prior art species share a useful property, this will generally be sufficient to motivate an ordinary artisan to make the claimed species. See MPEP 2144.06 , including MPEP 2144.06 4(d).

Therefore, the prior art did not need to rely upon the binding of LFA-1/Mac-1/p150,95, as currently claimed. Clearly, the prior art teaching of the HRV receptor would have either the inherent or expected properties of binding LFA-1/Mac-1/p150,95; given its ability to bind HRV.

With respect to the recitation of "artificial" does not appear; the patentability of a product does not depend on its method of production. In re Thorpe, 227 USPQ 964, 966 (Fed. Cir. 1985). See MPEP 2113.

Applicant's arguments are not found persuasive.

15. No claim is allowed.

16. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phillip Gambel whose telephone number is (703) 308-3997. The examiner can normally be reached Monday through Thursday from 7:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on (703) 308-3973. Any inquiry of a general nature or relating to the status of this application should be directed to the Technology Center 1600 receptionist whose telephone number is (703) 308-0196.

Papers related to this application may be submitted to Technology Center 1600 by facsimile transmission. Papers should be faxed to Technology Center 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center telephone number is (703) 305-3014.

Phillip Gambel

Phillip Gambel, PhD.
Primary Examiner
Technology Center 1600
December 14, 2000

FORM PTO-892 (REV. 2-92)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO.	GROUP ART UNIT	ATTACHMENT TO PAPER NUMBER
NOTICE OF REFERENCES CITED		08/474,382	1644	8
		APPLICANT(S) SPRING GER ET AL.		

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A						
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FOREIGN PATENT DOCUMENTS

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	L								
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OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

	R	1. COLONNO ET AL. VIRUS ATTACHMENT AND ENTRY INTO CELLS, PROCEEDINGS OF AN AIM CONFERENCE HELD IN PHILA. PA APRIL 10-13 1985. PAGES 109-115
	S	CROWELL ET AL. (EDS.) AMERICAN SOCIETY FOR MICROBIOLOGY WASHINGTON DC 1986.
*	S	2. TOMASSINI ET AL. PNAS 86: 4907-4911 (1989)
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GAMBEL	11/4/00	

* A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

Virus Attachment and Entry into Cells

Proceedings of an ASM Conference Held in Philadelphia, Pennsylvania, 10–13 April 1985

Editors:

Richard L. Crowell and Karl Lonberg-Holm

American Society for Microbiology
Washington, D.C. 1986

Characterization of the Cellular Receptor Specific for Attachment of Most Human Rhinovirus Serotypes

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Human rhinoviruses (HRVs) are members of the picornavirus family and are the major causative agent of the common cold in humans. Reciprocal competition binding studies have demonstrated that 20 of 24 serotypes of rhinoviruses compete for a single cellular receptor on HeLa cells. Using HeLa cells as an immunogen, we isolated a mouse monoclonal antibody which had the precise specificity predicted by the biological binding study. The receptor antibody is able to protect HeLa cells from infection by 78 of 88 HRV serotypes assayed. Pretreatment of cells with the antibody is also able to protect HeLa cells from infection by coxsackieviruses A11, A18, and A21, while showing no protective effect against a wide range of other viruses. The antibody demonstrates the same tissue tropism as the major group of HRVs, since it binds only to cells of human origin (with one exception, chimpanzee). Solubilization of HeLa cell membranes with detergents and subsequent chromatography on an immune affinity column resulted in the isolation of a 90,000-molecular-weight protein believed to be the major HRV cellular receptor protein.

Picornaviruses attach to animal cells by means of specific receptors on the cell surface (4, 7, 16). Frequently, the possession of appropriate cellular receptors is sufficient to determine the host range for a particular virus (6). Recent reviews (4, 11) of receptors utilized during picornavirus infections indicate that distinct receptor families exist for attachment of specific groups of picornaviruses. Receptor specificity has been further demonstrated by the isolation of monoclonal antibodies which specifically block the attachment of polioviruses (14) and group B coxsackieviruses (2). The human rhinovirus (HRV) family is composed of over 100 antigenically distinct serotypes and offers a unique opportunity to study receptor specificity (12).

HOW MANY HRV RECEPTORS ARE THERE?

This investigation was initiated to establish whether the large group of medically important viruses classified as HRV attach to susceptible cells by a similarly large variety of cellular receptors. Utilizing 10 HRV serotypes, Lonberg-Holm and his colleagues (9, 10) suggested that at least two different

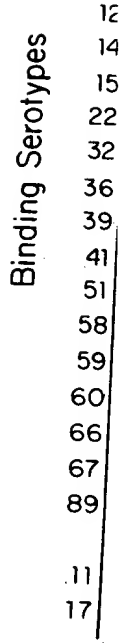
HRV receptors exist on HeLa cells. Further evidence for the existence of multiple HRV receptors was the ability of a limited number of HRV serotypes to attach to mouse-derived cells (15). Since the number of HRV serotypes examined in these earlier studies was limited, 24 randomly selected HRV serotypes were used in reciprocal competition binding assays to determine the total number of receptors utilized (1). The results from these assays are summarized in Fig. 1 and clearly define only two receptor groups. The first group contained 20 of the 24 serotypes which competed with one another for a single cellular receptor. A minor group, containing only 4 of the 24 HRV serotypes tested, shared a second and different cellular receptor. Members of this minor receptor group are also capable of binding to mouse L cells while the major group of viruses cannot (1).

Monoclonal antibodies were pursued to further explore the HRV cellular receptors. BALB/c mice were immunized with HeLa cells, and their spleen cells were fused to mouse myeloma cells. The resulting hybridoma cell supernatants were screened in a cell protection assay for their ability to protect cell monolayers from infection by HRVs (3). Of the thousands assayed, only one hybridoma cell culture supernatant was identified which could protect cells from HRV-14 infection. As a primary test of the monoclonal antibody's specificity, HeLa cells were treated with the antibody and challenged with each of the 24 serotypes represented in Fig. 1. The receptor monoclonal antibody appeared to have the precise specificity predicted by the biological study, since only the 20 HRV serotypes belonging to the major receptor group were unable to infect cells in the presence of the antibody (Table 1). The antibody was unable to protect cells from infection by the minor group serotypes, 1A, 2, 44, and 49.

Additional cell protection studies (Table 1) showed that the receptor antibody could not protect cells from infection by other picornaviruses or several other RNA and DNA viruses. Protection was observed in HeLa cells against infection by group A coxsackievirus serotypes 13, 18, and 21. This was not unexpected, since earlier studies (9) showed that coxsackievirus A21 and HRV-14 (major receptor group) competed for the same cellular receptor.

As a final test of the specificity of this receptor antibody, we surveyed 27 cell lines to determine whether the antibody demonstrated the same species tropism as the major group of HRV serotypes. Experiments involved parallel binding studies of radiolabeled HRV-15 and receptor monoclonal antibody. Results showed that the antibody binds only to cell lines to which the virus is capable of binding (3). With the exception of chimpanzee liver cells, specific binding occurred only to cells of human origin. Thus, the cellular receptor required for binding of the antibody to cell surfaces followed the same species tropism as the virus itself.

Convinced that the receptor monoclonal antibody was of the correct specificity, we assayed an additional 64 HRV serotypes against the antibody blockage. Results confirmed our original prediction that almost 90% of all HRV serotypes compete for a single receptor on cells, since the antibody was able to block infection by 78 of the 88 serotypes tested (Table 1). These data clearly confirmed that the ligand structure present on the viral capsid is highly conserved among HRV serotypes and represents the first defined structural link to this group of viruses.



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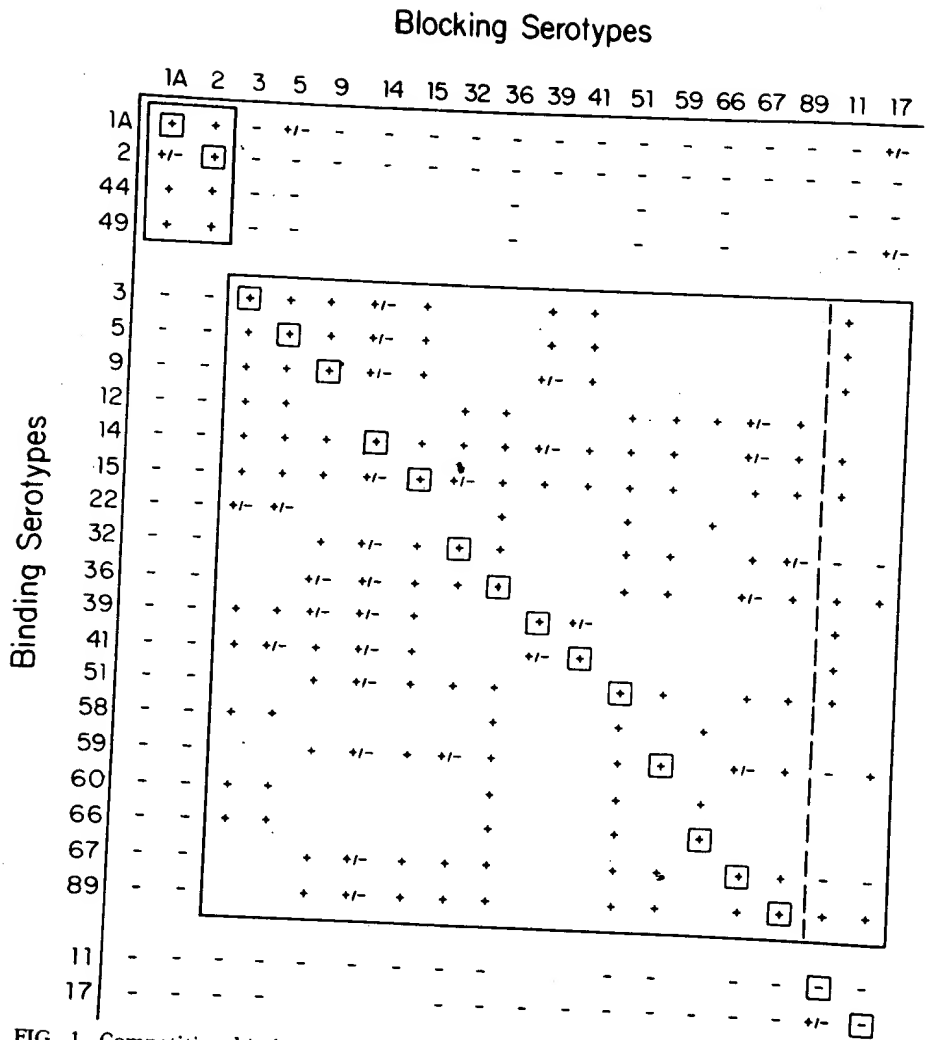

FIG. 1. Competition binding studies between 24 HRV serotypes. Twenty-four HRV serotypes were grown in HeLa R19 cells in the presence or absence of [^{35}S]methionine, the HRVs were purified, and titers were determined as previously described (1). Predetermined amounts of unlabeled virions of the serotypes listed across the top were used to saturate 2×10^5 to 3×10^5 HeLa cells prior to a challenge binding by the ^{35}S-labeled HRV serotypes listed on the left side (1). HRV serotypes which blocked at least 90% as effectively as the homologous virus were scored positive (+), those with which blocking was lower than 20% were scored negative (−), and intermediate percentages were scored +/− to indicate equivocal results. Boxed results are homologous competitions.

TABLE 1. Cell protection assay of receptor monoclonal antibody[a]

Result	Virus
Protection	HRV serotypes 3–28, 32–43, 45, 46, 48, 50–52, 54–61, 63–81, 83–86, 88, 89, and Hanks
	Coxsackieviruses A13, A18, and A21
No protection	HRV serotypes 1A, 1B, 2, 29–31, 44, 47, 49, and 62
	Polioviruses 1, 2, and 3
	Coxsackieviruses B2 and B3
	Echoviruses 1, 3, 6, and 20
	Hepatitis virus type A
	Vesicular stomatitis virus
	Newcastle disease virus
	Parainfluenza viruses 1, 2, and 3
	Influenza A virus
	Respiratory syncytial virus
	Vaccinia virus
	Adenoviruses 1 and 2

[a] Viruses, cells, and procedures used for infection of confluent monolayers have been described elsewhere (3). Assays were scored positive for protection if no cytopathic effect was evident in antibody-treated cultures versus untreated control cultures.

CHARACTERIZATION OF THE MAJOR HRV RECEPTOR

The mouse monoclonal antibody was determined to have an immunoglobulin G1 isotype by gel diffusion using isotype-specific antisera. The monoclonal antibody appears to have a very strong avidity for the receptor protein. Kinetics of binding showed that it binds to the receptor within 1 min, whereas HRVs require up to 1 h to achieve maximal binding (3). While the antibody is capable of blocking the attachment of virus to receptors, the virus is unable to effectively block the attachment of antibody. Although size differences may be involved, the greater affinity of the antibody for the receptor site appears to be the major explanation. To illustrate this point, ^{35}S-labeled HRV-14, HRV-15, and HRV-2 were bound to HeLa cell membranes and unbound virus was removed by washing. The membrane-virus complexes were then incubated with increasing amounts of monoclonal antibody, and the amount of virus released from the complex was measured. Results showed that up to 80% of HRV-14 (weak binder) and 35% of HRV-15 (strong binder) were released by antibody addition, while HRV-2 (minor group) showed no release from its receptor (3). This result strongly suggests that the antibody is capable of displacing previously bound virions of the major group of HRVs and will be a valuable tool in future studies on viral entry.

Since viruses have the capacity to mutate quite rapidly during propagation, we attempted to isolate viral mutants capable of overcoming the receptor antibody block. We first examined our viral stocks for the presence of natural variants. Antibody-treated and untreated confluent monolayers of HeLa cells were infected with 10^9 PFU (multiplicity of infection > 1,000) of HRV-2, HRV-14, HRV-15, and HRV-36, and plates were overlaid with medium (with or

without antibody) and 0.4% agar to restrict viral spread. After 3 days of incubation at 34°C, plates were stained to visualize the formation of plaques. The plates containing no antibody showed complete destruction of the cell monolayer by each of the four serotypes, as expected with such a massive amount of virus. However, not a single plaque was observed on the antibody-treated plates infected by HRV-14, HRV-15, and HRV-36 (3). The antibody-treated plate that was infected with HRV-2 was completely destroyed and served as a control. This result demonstrates that natural variants capable of bypassing this specific blockage do not preexist in our concentrated virus preparations.

A second experiment was then designed to select for these mutations. HRV-15 (100 to 200 PFU) was adsorbed to HeLa cell monolayers for 0.5, 2, 4, and 6 h to ensure eclipse of the virus particles prior to the addition of antibody in an agar overlay. After a 3-day incubation, no plaques were present in any of the antibody-treated plates regardless of when the antibody was added (3). While no direct mutagenesis has been attempted, these data strongly suggest that the viral ligand for the major HRV cellular receptor resides in a location that is not easily changed. In addition, this result clearly confirms that HRV transmission occurs only through receptor utilization.

Isolation and identification of the cellular receptor utilized by the major group of HRVs is a prerequisite to complete understanding of viral entry into cells. Solubilization of cellular receptors can be achieved by several methods, of which the most commonly used is detergent treatment. We have used several ionic and nonionic detergents in attempts to solubilize the HRV major receptor and have concluded that 0.3% sodium deoxycholate is the most efficient. Detergent-solubilized receptor preparations were chromatographed on an Affi-gel column coupled to receptor monoclonal antibody (5). After extensive washing, the bound material was eluted from the column with diethylamide and analyzed by polyacrylamide gel electrophoresis. Results (Fig. 2) showed a predominant protein band migrating with an apparent molecular weight of 90,000 (J. E. Tomassini and R. J. Colonno, submitted for publication). No equivalent protein of 90,000 molecular weight was found when Affi-gel columns without antibody were used or when receptors were solubilized from mouse L cells or other cell lines not containing the major HRV receptor. Further analysis of this candidate receptor protein is in progress.

The data presented here illustrate quite conclusively that the vast number (89%) of HRV serotypes utilize a single cellular receptor protein for attachment to susceptible cells. These data were obtained by studying competition for receptors among 24 randomly selected HRV serotypes and by isolation of a mouse monoclonal antibody capable of specifically blocking attachment of this major HRV group. Competition binding studies among the 10 HRV serotypes, belonging to the minor group, have demonstrated that these serotypes compete with one another for a second receptor that is clearly different from the major HRV receptor (3).

Studies with the receptor monoclonal antibody have repeatedly demonstrated the inability of the major group of HRVs to compensate for the receptor block. These results strongly suggest that the viral ligand resides at a location involved in the structural integrity of the capsid. Mutations in this region

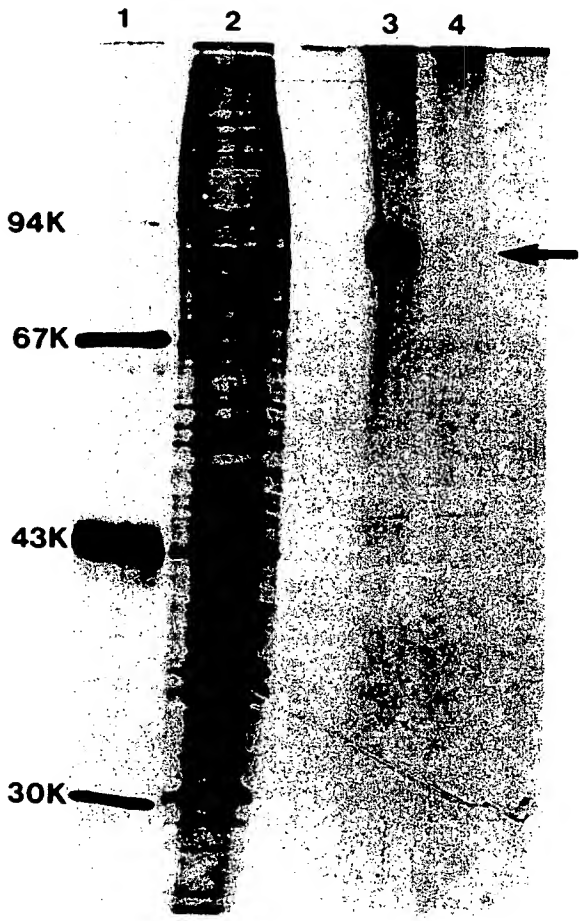

FIG. 2. Affinity chromatography of major HRV cellular receptor proteins. HeLa R19 cell membranes were solubilized with 0.3% sodium deoxycholate, and cellular debris was removed by high-speed centrifugation (Tomassini and Colonno, submitted). Solubilized supernatant was chromatographed on an Affi-gel column or an Affi-gel column containing linked receptor monoclonal antibody (5). The columns were washed extensively in the presence of 0.3% sodium deoxycholate. Bound material was eluted with 0.2% sodium dodecyl sulfate and analyzed on a 10% polyacrylamide-sodium dodecyl sulfate gel (8). The gel was fixed and proteins were visualized by silver staining (13). Lane 1, Protein markers; lane 2, high-speed supernatant; lane 3, Affi-gel with antibody; lane 4, Affi-gel without antibody. Arrow indicates position of receptor protein.

probably result in an instability of the capsid structure and are, therefore, lethal mutations.

While we have isolated a cellular protein involved in HRV attachment, we know little about its normal function or the number of components required to make up an active HRV receptor site. The viral attachment site does not appear to be an essential structure on the HeLa cell surface, since prolonged treatment with the receptor monoclonal antibody results in no inhibition of cell growth or DNA, RNA, and protein synthesis. It is hoped that further characterization and cloning of the receptor protein will provide a better understanding of this important cellular receptor.

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